JC09 Rec'd PCT/PTO 16 MAR 2001

FORM PTO-1390 (REV 10-2000) | U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE | ATTORNEY'S DOCKET NUMBER: FJIN-107

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

U.S. APPLICATION NO. (If known, see 37 CFR 1.5): 09/787397

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/JP99/05080	September 17, 1999	September 17, 1998

TITLE OF INVENTION: PREVENTIVE AND/OR THERAPEUTIC FOR OBESITY

APPLICANT(S) FOR DO/EO/US: Masaaki Goto, Akihiro Tomoyasu, Kyoji Yamaguchi, Masahiko Kinosaki, Nobuaki Nakagawa

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. [x] This is a submission of items concerning a filing under 35 U.S.C. 371.
2. [ ] This is a or submission of items concerning a filing under 35 U.S.C. 371.
3. [x] This is an express request to promptly begin national examination procedures (35 U.S.C. 371(f)).
4. [ ] The US has been elected by the expiration of 19 months from the priority date (PCT Article 31).
5. [x] A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. [ ] is attached hereto (required only if not communicated by the International Bureau).
   b. [x] has been communicated by the International Bureau.
   c. [ ] is not required, as the application was filed in the United States Receiving Office (RO/US).
6. [x] An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).
7. [x] Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. [ ] are attached hereto (required only if not communicated by the International Bureau).
   b. [ ] have been communicated by the International Bureau.
   c. [ ] have not been made; however, the time limit for making such amendments has NOT expired.
   d. [x] have not been made and will not be made.
8. [ ] An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. [x] An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)). (unexecuted)
10. [ ] An English language translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).
11. [x] An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. [ ] An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. [x] A FIRST preliminary amendment.
    [ ] A SECOND or SUBSEQUENT preliminary amendment.
14. [ ] A substitute specification.
15. [ ] A change of power of attorney and/or address letter.
16. [x] Other items or information:
    a. Copy of Request Form (PCT/RO/101) in Japanese and English (8 pages)
    b. Statement Regarding Sameness and New Matter with Regards to the CRF and the Substitute Sequence Listing
    c. Substitute Sequence Listing Disk in computer readable form
    d. Substitute Sequence Listing
    e. Copy of International Search Report dated December 21, 1999
    f. International Deposit Receipt Form BP-6736 in Japanese and English
    g. English translation of International Preliminary Examination Report

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U.S. APPLICATION NO (if known, see 37 CFR 1.5) 09/787397 | INTERNATIONAL APPLICATION NO PCT/JP99/05080 | ATTORNEY'S DOCKET NUMBER FJIN-107

17. ☒ The following fees are submitted:	CALCULATIONS	PTO USE ONLY
BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)):		
Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO **$1000.00**		
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO **$860.00**		
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO **$710.00**		
International preliminary examination fee paid to USPTO (37 CFR 1.482) but all claims did not satisfy provisions of PCT Article 33(1)-(4) **$690.00**		
International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(1)-(4) **$100.00**		
ENTER APPROPRIATE BASIC FEE AMOUNT =	$ 860.00	
Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492(e)).	$	

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total claims	- 20 =		X **$18.00**	$	
Independent claims	- 3 =		X **$80.00**	$	
MULTIPLE DEPENDENT CLAIM(S) (if applicable)			+ **$270.00**	$	
TOTAL OF ABOVE CALCULATIONS =				$ 860.00	
☐ Applicant claims small entity status. See 37 CFR 1.27. The fees indicated above are reduced by 1/2.				$	
SUBTOTAL =				$ 860.00	
Processing fee of **$130.00** for furnishing the English translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492(f)). +				$	
TOTAL NATIONAL FEE =				$ 860.00	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). **$40.00** per property +				$	
TOTAL FEES ENCLOSED =				$ 860.00	
				Amount to be refunded:	$
				charged:	$

a. ☒ A check in the amount of $ 860.00 to cover the above fees is enclosed.

b. ☐ Please charge my Deposit Account No. _____ in the amount of $_____ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 501032. A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO.

Barry I. Hollander, Esq.
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Date: March 16, 2001

Barry I. Hollander

SIGNATURE

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REGISTRATION NUMBER

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

#3/a

Applicant	:	GOTO et al.	
Serial No.	:	U.S. National Stage Application Of PCT/JP99/05080	Group Art Unit:
Filed	:	Concurrently herewith	Examiner:
Title	:	PREVENTIVE AND/OR THERAPEUTIC FOR OBESITY	

PRELIMINARY AMENDMENT

Assistant Commissioner for Patents
Washington, DC 20231

Sir:

With reference to the above-identified patent application, please amend the application prior to issuance of the first Office Action:

IN THE SPECIFICATION

Please replace the Sequence Listing in the English translation of PCT/JP99/05080, as filed, with the Substitute Sequence Listing filed concurrently herewith.

IN THE CLAIMS

Please add new claims 2-20, as follows:

2. A preventive and/or therapeutic agent for obesity according to claim 1 wherein said stanniocalcin is obtained from a recombinant expression cell.

3. A preventive and/or therapeutic agent for obesity according to claim 2 wherein

said cell is an animal cell.

4. A preventive and/or therapeutic agent for obesity according to claim 1 which is administered to human beings and animals.

5. A preventive and/or therapeutic agent for obesity according to claim 1 in the form of a pharmaceutical composition.

6. A preventive and/or therapeutic agent for obesity according to claim 5 wherein said pharmaceutical composition is formulated for oral or parenteral administration.

7. A preventive and/or therapeutic agent for obesity according to claim 5 wherein said pharmaceutical composition is selected from the group consisting of compositions for injection, compositions for dripping, suppositories, nasal agents, sublingual agents and percutaneous absorption agents.

8. A preventive and/or therapeutic agent for obesity according to claim 5 wherein said pharmaceutical composition comprises pharmaceutically acceptable carriers, excipients, stabilizers, coloring matters, or surfactants.

9. A preventive and/or therapeutic agent for obesity according to claim 5 wherein said pharmaceutical composition is a composition formulated for injection comprising a pharmacologically effective amount of stanniocalcin and at least one pharmaceutically acceptable excipient and/or activator.

10. A preventive and/or therapeutic agent for obesity according to claim 9 wherein said excipient and/or activator is one or more of amino acids, human serum

albumin, sugars and/or cellulose derivatives.

11. A preventive and/or therapeutic agent for obesity according to claim 1 which is administered at a daily dose of 10 μg to 10 mg/kg body weight.

12. A preventive and/or therapeutic agent for obesity according to claim 6 which is administered intravenously.

13. A method for treating or preventing obesity which comprises administering to humans or animals a pharmaceutically effective amount of stanniocalcin in the form of a pharmaceutical composition.

14. A method for treating or preventing obesity according to claim 13 wherein said pharmaceutical composition is administered orally or parenterally.

15. A method for treating or preventing obesity according to claim 13 wherein said pharmaceutical composition is in a form selected from the group consisting of compositions for injection, compositions for dripping, suppositories, nasal agents, sublingual agents and percutaneous absorption agents.

16. A method for treating or preventing obesity according to claim 13 wherein said pharmaceutical composition further comprises pharmaceutically acceptable carriers, excipients, stabilizers, coloring matters, or surfactants.

17. A method for treating or preventing obesity according to claim 13 wherein said pharmaceutical composition is a composition formulated for injection comprising a pharmacologically effective amount of stanniocalcin and at least one pharmaceutically

acceptable excipient and/or activator.

18. A method for treating or preventing obesity according to claim 17 wherein said excipient and/or activator comprise amino acids, human serum albumin, sugars, and/or cellulose derivatives.

19. A method for treating or preventing obesity according to claim 13 wherein said pharmaceutical composition is administered at a daily dose of 10 μg to 10 mg/kg body weight.

20. A method for treating or preventing obesity according to claim 13 wherein said pharmaceutical composition is administered intravenously.

Support for the Amendment

Support for the Substitute Sequence Listing, filed concurrently herewith, can be found, for example, in the Sequence Listing in the English Translation of PCT/JP99/05080, as filed, at pages 1-5. Amendments to the Sequence Listing are solely editorial in nature. The Substitute Sequence Listing contains no new matter.

Support for new claims 2 and 3 can be found, for example, in the instant specification at page 5, lines 11-17. Support for new claims 4, 5 and 13 can be found, for example, in the instant specification at page 6, lines 3-6. Support for new claims 6 and 14 can be found, for example, in the instant specification at page 6, lines 7-8. Support for new claims 7 and 15 can be found, for example, in the instant specification at page 6, lines 8-12. Support for new claims 8 and 16 can be found, for example, in the instant specification at page 6, lines 12-16. Support for new claims 9 and 17 can be found, for example, in the instant specification at page 6, lines 16-22. Support for new claims 10 and 18 can be found, for example, in the instant specification at page 6, lines 16-22, and page 14, lines 12-20. Support for new claims 11 and 19 can be found, for example, in the instant specification at page 7, lines 3-5. Support for new claims 12 and 20 can be found, for example, in the instant specification at page 7, lines 5-7. Upon the entry of this Preliminary Amendment, claims 1-20 will stand pending in the instant application. No new matter is added by the instant Amendment.

Based on the above, an early and favorable action on the merits is earnestly

solicited.

Filed concurrently herewith, Applicants have submitted a Substitute Sequence Listing and a diskette in a computer-readable form.

If any fee is due, please charge our deposit account no. 501032.

Respectfully submitted,

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March 16, 2001

Enclosure: "Versions with Markings to Show Changes Made"

Version with Markings to Show Changes Made

New claims 2-20 have been added.

世界知的所有権機関
国際事務局

PCT

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添付公開書類
国際調査報告書

(54)Title: **PREVENTIVES AND/OR REMEDIES FOR OBESITY**

(54)発明の名称 肥満予防及び／又は治療剤

(57) Abstract

Novel preventives and/or remedies for obesity. These preventives and/or remedies contain as the active ingredient stanniocalcin. Because of having an excellent activity of inhibiting the differentiation and maturation of adipocytes, they are useful as drugs for preventing and/or treating obesity.

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DESCRIPTION

PREVENTIVE AND/OR THERAPEUTIC FOR OBESITY

Technical Field

The present invention relates to a novel preventive and/or therapeutic for obesity.

A pharmaceutical preparation of the present invention has excellent preventive and/or therapeutic effects for obesity and is useful as a pharmaceutical.

Background Art

Obesity is a risk factor of diseases such as diabetes mellitus, hypertonia, and heart disease, which threaten health of people in advanced countries. Obesity means physical conditions wherein adipose tissues have abnormally accumulated. Adipose tissues are special organs wherein surplus in vivo energies are stored as fat or triglyceride, and constructed of fibroblasts including adipocytes and their precursors, macrophages, blood vessel surrounding cells, blood cells, and the like.

Adipocytes are said to amount from 1/3 to 2/3 of cells which are present in adipose tissues and to accumulate fats or triglycerides therein. Adipocytes differentiate and mature through the process starting from mesenchymal multipotent stem cells, and growing into lipoblasts which have acquired a base as adipocytes, precursor adipocytes with no lipid droplets but

having initial markers of adipocytes, immatured adipocytes containing lipid droplets, and finally into matured adipocytes containing a large quantity of accumulated fats. Adipocytes of adults suffering from slight obesity hypertrophically grow due to increase in the amount of accumulated triglyceride. Number of adipocytes increases as the degree of obesity becomes conspicuous. Therefore, decreasing the number of adipocytes by controlling differentiation and maturation or suppressing hypertrophia of matured adipocytes are expected to stop progress of obesity by suppressing the increase in the amount of accumulated fats, and to treat obesity. Control of in vivo adipocyte differentiation has been proven to undergo either positively or negatively according to a number of factors derived from environmental factors such as ingestion, exercise, and so on. As cytokines which control differentiation of adipocytes from adipocyte precursors, tumor necrosis factor-α (TNF-α: Torti F. M. et al., Science, Vol. 229, p 867 (1985)), transforming growth factor-β (Ignotz R. A. et al., Proc. Natl. Acad. Sci. USA, Vol. 82, p 8530 (1985)) , preadipocyte factor-1 (Pref-1: Smas C.M. et al., Cell, Vol. 73, p 725 (1993)) , and the like have been reported. In addition, leptin, the translational product of an ob gene which has recently been cloned, has been reported to possibly decrease the intake amount and the weight of adipose tissues via central nerve

system (Levin N. et al. Proc. Natl. Acad. Sci. USA, Vol. 93. P 1726, 1996) .

Furthermore, intracerebral peptide-neuropeptide Y which exhibits a strong appetite stimulating effect and its receptor are gathering attention as materials for the development of an obesity suppressing pharmaceutical (Sainsburg A. et al, Diabetologia, Vol.39, p353, 1996). These cytokines are expected to become a therapeutic agent for obesity due to their adipocyte depressing action on accumlation of fat. Clinical tests as an obesity therapeutic or preventive agent is ongoing on some of these cytokines such as leptin.

At present, one obesity therapeutic or preventive agent is commercially available in the USA under the Redux™ (American Home Products Co.). Other drugs such as Meridia (Kunol Co.) and Xenical (Roche Co.) will be approved as an obesity treating agent or a fat absorption inhibitor in the USA. The treatments method using these pharmaceuticals, however, are not necessarily satisfactory in the effects and therapeutic results. Development of a new agent which is available exhibits for these pharmaceuticals higher curative effect and less side effect usable have been desired.

Disclosure of the Invention

In view of the above circumstances, the present inventors have intensive investigated a substance which shows

anti-obesity activity or obesity-curing activity, and as a result, found that stanniocalcin (STC: Olsen H.S. et al., Proc. Natl. Acad. Sci. USA, vol. 93, p 1792 (1996)) which is known as a protein controlling methabolism of minerals, exhibits adipogenesis inhibitory activity, or inhibitory activity of differentiation and/or maturation of adipocytes, which physiologic activity has not been expected of stanniocalcin at all in the past. Accordingly, the object of the present invention is to provide a preventive and/or therapeutical agent for obesity containing a novel substance as an effective ingredient. The present invention relates to a preventive and/or therapeutical agent for obesity, which contains stanniocalcin as an effective ingredient. The pharmaceutical preparation according to the present invention can exhibit excellent preventive and/or therapeutic agent effects for obesity and are useful as a pharmaceutical.

Stanniocalcin was discovered in fishes at first and was subsequently clarified to exist in mammals including humans. Then, cDNA of human embryo was isolated by the genetic engineering procedure on the basis of structural similarity. Human stanniocalcin can be obtained by expressing the resultant cDNA in a variety of cells using the genetic engineering technique.

It has been well known that stanniocalcin reduces a calcium level *in vivo* when given to fish, and also inhibits phosphate excretion to urine when administered to rats (Proc. Natl. Aca. Sci. USA., 93, 1792 (1996)). However, stanniocalcin has not been known to possess excellent preventive and therapeutic effects for obesity.

The Best Mode for Carrying out the Invention

Stanniocalcin, or the effective ingredient according to the present invention, can be obtained by the method of Olsen H. S. et al. (Proc. Natl. Acad. Sci. USA, vol. 93, p 1792 (1996)). Specifically, the above-described literature reference or gene bank or the like can be searched to learn the sequence of cDNA of stanniocalcin, and based on the sequence information, stanniocalcin cDNA can be obtained using the PCR method etc.. The stanniocalcin expression cell can be obtained by transfections of the expression vector into animal cells etc., the said expression vector is obtained by insertion of the resultant cDNA. Then, stanniocalcin can be obtained by cultivating the resultant stanniocalcin expression cells, followed by purification of the resultant culture solution by conventionally employed procedures. The adipogenesis inhibitory activity can be determined by estimating the suppression effects of adipogenesis induced by dexamethasone with retardation of triglyceride accumulation using mouse preadipocytic cell as a target according to the

method of Kodama H. et al. (Journal of Cellular Physiology, Vol. 112, p83 (1982)),

Stanniocalcin, or the effective ingredient of the present invention, can be safely administered to human being and animals in the form of pharmaceutical compositions intended for use in the prevention and/or treatment of obesity. Stanniocalcin can be made into pharmaceutical preparations and administered either for orally or parenterally. Examples of the pharmaceutical composition include compositions for injection, compositions for dripping, suppositories, nasal agent, sublingual agent, percutaneous absorption agent, and the like. These pharmaceutical preparations are formulated according to known pharmaceutical preparation methods using pharmaceutically acceptable carriers, excipients, stabilizers, coloring matters, surfactants and other additives, and made into target preparations. In the case of compositions for injection, a pharmacologically effective amount of stanniocalcin, which is the effective ingredient of the present invention, may be mixed with pharmaceutically acceptable excipients/activators, such as amino acids, sugars, cellulose derivatives and other organic/inorganic compounds, which may be generally added to compositions for injection. If necessary, pH adjusting agents, buffer agents, stabilizers, solubilizing agents, etc. may be added to thereby make a

variety of injectable solutions in accordance with the conventional procedures.

Administration thereof is normally done to human adults at a daily dose of 10 μg to 10 mg/kg body weight, as divided in several times, either orally or parenterally. The particularly preferred dosage form is intravenous administration.

Example

Examples given in the below describe the present invention in more detail, whereby these examples are merely illustrative, and the present invention is in no way understood to be limited by them.

Example 1

Production of stanniocalcin

i) Isolation of poly(A)+ RNA from IMR-90 cells (pulmonary fibroblasts of human embryo, ATCC CCL-186)

About 10 μg of poly(A)+ RNA was isolated from 1×10^8 of IMR-90 cells using Fast Track mRNA Isolation Kit (Invitrogen Inc.) according to the protocol of Invitrogen Inc..

ii) Construction of human stanniocalcin expression vector

A single-stranded cDNA was synthesized using Super-Script II cDNA synthesis Kit (Gibco BRL Inc.) and 1 μg of the isolated poly(A)+ RNA used as a template, according to the protocol of Gibco BRL Inc.. Stanniocalcin (STC) cDNA fragment

was obtained by carrying out PCR using the obtained cDNA template and primer STCF1N (Sequence Identification No. 1) and primer STCR1Xh (Sequence Identification No. 2) as designed according to the nucleotide sequence of human stanniocalcin. The composition for PCR solution is as follows:

10X Ex Taq Buffer (Takara Shuzo Co.)	10 µl
2.5 mM dNTP	8 µl
cDNA solution	1 µl
Ex Taq (Takara Shuzo Co.)	0.5 µl
Distilled water	74.5 µl
20 µM Primer STCF1N	5 µl
100 µM Primer STCR1Xh	1 µl

The above-described solutions were mixed in a microcentrifugal tube, and PCR was performed under the following conditions: after pretreatment at 95°C for 3 min, the reaction of the three steps of at 95°C for 30 sec, at 55°C for 30 sec and at 72°C for 2 min was repeated 30 times. Then, the reaction mixture incubated at 70°C for 5 min. A portion of the reaction mixture was subjected to agarose gel electrophoresis, and a uniform DNA fragment of about 900 bp was identified. The fragment was sequenced by the conventional method, and it was confirmed to obtain the cDNA encoding stanniocalcin gene. The cDNA sequence and the amino acid sequence are shown in

Sequence Identification Nos. 3 and 4, of sequence table respectively.

The resultant DNA fragment of about 900 bp was purified using QIAEXII DNA extraction kit (QIAGEN Inc.), and the purified DNA was cleaved by restriction enzymes XhoI and NheI (Takara Shuzo Co.) and purified using QIAEXII DNA extraction kit (STC XhoI-NheI fragment). Plasmid pCEPSTC which contained DNA encoding stanniocalcin gene was obtained by ligating the STC XhoI-NheI fragment to pCEP4 (Invitrogen Inc.) cleaved by restriction enzymes XhoI and NheI by ligation kit ver. 2 (Takara Shuzo Co.). *E. coli* (DH5 α ; Gibco BRL Inc.) containing the plasmid has been deposited, in the name of DH5α/pCEP-STC and under Accession No. FERM BP-6736 in National Institute of Bioscience and Human-Technology, Agency of Industrial Science and Technology, The Ministry of International Trade and Industry, located at 1-3, Higashi 1-chome, Tsukuba-shi, Ibaraki-ken, Japan (postal code 305-8566) on May 31, 1999. No erroneous uptake of bases in the DNA portion derived from PCR during the DNA synthesis was meanwhile confirmed by DNA sequencing.

iii) Expression of human stanniocalcin

E. coli DH5α having pCEPSTC as obtained in Example 1-ii) was cultivated with shaking in 2 ml of Teriffic Broth (Life Technologies Inc.) containing 50 μg/ml of ampicillin (Sigma

Inc.) and 4.7 % of glycerol overnight at 37°C, and the plasmid DNA was purified from the bacterial cells using QIAWELL kit (QIAGEN Inc.). 293-EBNA cells (Invitrogen Inc.) in IMDM (Life Technologies Inc.) containing 10 % of fetal bovine serum were seeded in each well of a 24-well plate to 2×10^5 /well/ml, followed by cultivation in a CO_2 incubator (5 % CO_2) at 37°C overnight. pCEPSTC or pCEP4 was transfected to 293-EBNA cells using Fugene 6 (Behringer Mannheim Co.). DNA and Fugene 6 were used in portions of 0.5 μg and 1 μl, respectively. After transfection, the transfected cells were cultivated in a CO_2 incubator (5 % CO_2) at 37°C for 3 days. The resultant culture solution was assayed for adipogenesis inhibitory activity by the below-described procedure.

iv) Determination of adipogenesis inhibitory activity

Adipogenesis inhibitory activity was determined by the following procedure according to the method of Kodama H. et al. (Journal of Cellular Physiology, Vol. 112, p 83 (1982)): that is, using mouse pre-adipocytic cell strain MC3T3-G2/PA6 (RIKEN GENE BANK, RCB1127) as a target cell, the adipogenesis induced by dexamethasone was determined with triglyceride accumulation as an index of its inhibitory activity. The culture solution from the sample (Example 1-iii)) diluted with α-MEM (Gibco BRL Inc.) containing 10 % of fetal bovine serum, the culture solution of cells having the vector alone transfected, and the

culture solution of pure 293-EBNA cells were distributed in each portion of 50 μl into 96-well microplate, respectively, and 3×10^3 cells of pre-adipocytic cell strain MC3T3-G2/PA6 after being suspended in 50 μl of α-MEM containing 2×10^{-7} M of dexamethasone and 10 % of fetal bovine serum were seeded, followed cultivation at 5% CO_2, 37°C and 100% humidity for one week. After cultivation for 7 days, the culture medium was removed by aspiration, then air-dried and assayed for the triglyceride accumulated in adipocytes with use of a triglyceride measuring kit (Triglyceride G-Test Wako, Code No. 274-69802, Wako Pure Chemicals Ind. Co.). The decreases at OD 510 nm were used for assessment of adipogenesis inhibitory activity. The obtained results are shown in Table 1. As the result, stanniocalcin in the resultant culture solution was confirmed to exhibit adipogenesis inhibitory activity.

Table 1:

Dilution	1/4	1/8	1/16	1/32
Culture solution of STC gene-transfected cells	0.061	0.060	0.057	0.054
Culture solution of vector-transfected cells	0.036	0.021	0.009	0.007
Culture solution of	0.032	0.017	0.014	0.011

293-EBNA cells

Example 2

Determination of adipogenesis inhibitory activity using cells of mouse preadipocytic cell strain 3T3/L1

Using mouse pre-adipocytic cell strain 3T3-L1 (deposited at ATCC-Accession No. CL173) as a target, the formation of adipocytes induced by dexamethasone and 1-methyl-3-isobutylxanthine was measured by means of triglyceride accumulation, to determine the suppressing activity against adipocyte formation. Specifically, 50 µl of a sample equivalent to the one in Example 1 diluted with α-MEM (Gibco BRL Inc.) containing 10 % of fetal bovine serum was placed into a 96-well microplate, and 5×10^3 cells of mouse pre-adipocyte 3T3-LI were suspended in 50 µl of α-MEM containing 4×10^{-7} M of dexamethasone, 2×10^{-5} M of 1-methyl-3-isobutylxanthine and 10 % of fetal bovine serum and then seeded, followed by cultivation at 5% CO_2, 37°C and 100% humidity for one week. After cultivation for 7 days, the culture medium was removed by aspiration, and the cells were air-dried to measure the triglyceride accumulated in adipocytes using a triglyceride assay kit (Triglyceride G-Test Wako, Code No. 274-69802, Wako Pure Chemicals Ind. Co.). The

decrease of OD at 510 nm was taken as adipogenesis inhibitory activity. The obtained results are shown in Table 2. As a result stanniocalcin in the culture solution was confirmed to exhibit adipogenesis inhibitory activity, as in Example 1, when 3T3-L1 cells are used as a target.

Table 2:

Dilution	1/4	1/8	1/16	1/32
Culture solution of STC gene-transfected cells	0.081	0.083	0.082	0.083
Culture solution of vector-transfected cells	0.026	0.017	0.012	0.011
Culture solution of 293-EBNA cells	0.021	0.004	0.006	0.016

Example 3

Pharmaceutical Preparation Examples

Pharmaceutical Preparation Example 1: Production of Injection preparation

One mg of stanniocalcin obtained in Example 1 and 50 mg of human serum albumin were dissolved in 100 ml of 0.01M phosphate buffer solution (PBS, pH 7.0), and the solution was sterilized, divided into vials (2 ml each), lyophillized and sealed.

Pharmaceutical Preparation Example 2: Production of Injection preparation.

Fifty mg of stanniocalcin obtained in Example 1, 1 mg of Tween 80 and 50 mg of human serum albumin were dissolved in

100 ml of 0.01M phosphate buffer solution (PBS, pH 7.0), and the solution was sterilized, divided into vials (2 ml each), lyophillized and sealed.

Pharmaceutical Preparation Example 3: Production of Injection preparation.

One hundred mg of stanniocalcin obtained in Example 1, 50 mg of human serum albumin and 4 g of sorbitol were dissolved in 20 ml of 0.01M phosphate buffer solution (PBS, pH 7.0), and the solution was sterilized, divided into vials, lyophillized and sealed.

Industrial Applicability

According to the present invention, there is provided a novel preventive and/or therapeutic for obesity, which contains stanniocalcin as an effective ingredient. The pharmaceutical preparation of the present invention can exhibit excellent preventive and/or therapeutic effects against obesity and is useful as a pharmaceutical.

Reference to the Deposited Microorganisms

a. Name and address of the Depositary organization in which the relevant microorganisms were deposited:

Name: National Institute of Bioscience and Human-Technology, Agency of Industrial Science and Technology, The Ministry of International Trade and Industry

Address: 1-3 Higashi 1-chome, Tsukuba-shi, Ibaraki-ken, Japan (postal code 305-8566)

b. The date when deposit was made with the organization of a. May 31, 1999 (as transferred from Bikoken No. P-16933, which was deposited on August 11, 1998).

c. Accession Number attached to the deposit by the organization of a.

FERM BP-6736

CLAIM

1. A preventive and/or therapeutic agent for obesity which contains stanniocalcin as an effective ingredient.

ABSTRACT

To provide a novel preventive and/or therapeutic agent for obesity.

The preventive and/or therapeutic agent for obesity contains as an active ingredient stanniocalcin.

The preventive and/or therapeutic agent of the present invention has an excellent activity of inhibiting the differentiation and maturation of adipocytes, thereby being useful as a drug for preventing and/or treating obesity.

#6

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DECLARATION FOR UTILITY OR DESIGN PATENT APPLICATION (37 CFR 1.63)

☐ Declaration Submitted with Initial Filing **OR** ☒ Declaration Submitted after Initial Filing (surcharge (37 CFR 1.16 (e)) required)

Attorney Docket Number	FJIN-107
First Named Inventor	GOTO
COMPLETE IF KNOWN	
Application Number	09 / 787,397
Filing Date	03/16/2001
Group Art Unit	
Examiner Name	

As a below named inventor, I hereby declare that:

My residence, post office address, and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled

PREVENTIVE AND/OR THERAPEUTIC FOR OBESITY

(Title of the Invention)

the specification of which
☐ is attached hereto
OR
☒ was filed on (MM/DD/YYYY) 03/16/2001 as United States Application Number or PCT International Application Number 09/787,397 and was amended on (MM/DD/YYYY) 03/16/2001 (if applicable)

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment specifically referred to above

I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR 1.56.

I hereby claim foreign priority benefits under 35 U S C 119(a)-(d) or 365(b) of any foreign application(s) for patent or inventor's certificate, or 365(a) of any PCT international application which designated at least one country other than the United States of America, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or of any PCT international application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application Number(s)	Country	Foreign Filing Date (MM/DD/YYYY)	Priority Not Claimed	Certified Copy Attached? YES	Certified Copy Attached? NO
263004/1998	Japan	09/17/1998	☐	☐	☒
			☐	☐	☐
			☐	☐	☐
			☐	☐	☐

☐ Additional foreign application numbers are listed on a supplemental priority data sheet PTO/SB/02B attached hereto:

I hereby claim the benefit under 35 U S C 119(e) of any United States provisional application(s) listed below

Application Number(s)	Filing Date (MM/DD/YYYY)

☐ Additional provisional application numbers are listed on a supplemental priority data sheet PTO/SB/02B attached hereto.

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DECLARATION — Utility or Design Patent Application

I hereby claim the benefit under 35 U.S.C. 120 of any United States application(s), or 365(c) of any PCT international application designating the United States of America, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of 35 U.S.C. 112, I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR 1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application

U.S. Parent Application or PCT Parent Number	Parent Filing Date (MM/DD/YYYY)	Parent Patent Number (if applicable)
PCT/JP99/05080	09/17/1999	

☐ Additional U.S. or PCT international application numbers are listed on a supplemental priority data sheet PTO/SB/02B attached hereto

As a named inventor, I hereby appoint the following registered practitioner(s) to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith ☐ Customer Number [ ] → *Place Customer Number Bar Code Label here*
OR
[x] Registered practitioner(s) name/registration number listed below

Name	Registration Number	Name	Registration Number
Barry I. Hollander	28,566	Andrew E. C. Merriam	47,268

☐ Additional registered practitioner(s) named on supplemental Registered Practitioner Information sheet PTO/SB/02C attached hereto

Direct all correspondence to: ☐ Customer Number or Bar Code Label [023290] *OR* [x] Correspondence address below

Name	Barry I. Hollander				
Address	Hollander Law Firm, P.L.C.				
Address	Suite 305, 10300 Eaton Place				
City	Fairfax	**State**	VA	**ZIP**	22030
Country	United States	**Telephone**	703-383-4800	**Fax**	703-383-4804

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. 1001 and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Name of Sole or First Inventor: ☐ A petition has been filed for this unsigned inventor

1-00

Given Name (first and middle [if any])	Family Name or Surname
Masaaki	Goto

Inventor's Signature	Masaaki Goto					**Date**	4/27/2001
Residence: City	Shimotsuga-gun, Tochigi	**State**	JPX	**Country**	Japan	**Citizenship**	Japan
Post Office Address	456-1, Shimokoyama, Ishibashi-machi						
Post Office Address							
City	Shimotsuga-gun, Tochigi	**State**		**ZIP**	329-0511	**Country**	Japan

[x] Additional inventors are being named on the 2 supplemental Additional Inventor(s) sheet(s) PTO/SB/02A attached hereto

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ADDITIONAL INVENTOR(S) Supplemental Sheet
Page 1 of 2

2-00

Name of Additional Joint Inventor, if any:	☐ A petition has been filed for this unsigned inventor							
Given Name (first and middle [if any])				Family Name or Surname				
Akihiro				Tomoyasu				
Inventor's Signature	Akihiro Tomoyasu						**Date**	4/27, 2001
Residence: City	Shimotsuga-gun, Tochigi	**State**	JPX	**Country**	Japan		**Citizenship**	Japan
Post Office Address	SK-Mansion 3-E, 1-3-3, Omatsuyama, Ishibashi-machi							
Post Office Address								
City	Shimotsuga-gun, Tochigi	**State**		**ZIP**	329-0519	**Country**	Japan	

3-00

Name of Additional Joint Inventor, if any:	☐ A petition has been filed for this unsigned inventor							
Given Name (first and middle [if any])				Family Name or Surname				
Kyoji				Yamaguchi				
Inventor's Signature	Kyoji Y.						**Date**	4/27, 200
Residence: City	Omiya-shi, Saitama	**State**	JPX	**Country**	Japan		**Citizenship**	Japan
Post Office Address	Lionsgarden Higashiomiya 1-524, 702-12 Shimacho							
Post Office Address								
City	Omiya-shi, Saitama	**State**		**ZIP**	330-0006	**Country**	Japan	

4-00

Name of Additional Joint Inventor, if any:	☐ A petition has been filed for this unsigned inventor							
Given Name (first and middle [if any])				Family Name or Surname				
Masahiko				Kinosaki				
Inventor's Signature	Masahiko Kinosaki						**Date**	4/27, 001
Residence: City	Kawachi-gun, Tochigi	**State**	JPX	**Country**	Japan		**Citizenship**	Japan
Post Office Address	53-8, Yuukigaoka, Kaminokawa-machi							
Post Office Address								
City	Kawachi-gun, Tochigi	**State**		**ZIP**	329-0528	**Country**	Japan	

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DECLARATION

ADDITIONAL INVENTOR(S)
Supplemental Sheet
Page 2 of 2

5-00

Name of Additional Joint Inventor, if any:	☐ A petition has been filed for this unsigned inventor							
Given Name (first and middle [if any])			Family Name or Surname					
Nobuaki			Nakagawa					
Inventor's Signature	Nobuaki Nakagawa					**Date**	4/27/2001	
Residence: City	Shimotsuga-gun, Tochigi	**State**	JPX	**Country**	Japan	**Citizenship**	Japan	
Post Office Address	Heights Sakae E201, 31-1, Kawanago, Kokubunji-machi							
Post Office Address								
City	Shimotsuga-gun, Tochigi	**State**		**ZIP**	329-0415	**Country**	Japan	

Name of Additional Joint Inventor, if any:	☐ A petition has been filed for this unsigned inventor							
Given Name (first and middle [if any])			Family Name or Surname					
Inventor's Signature						**Date**		
Residence: City		**State**		**Country**		**Citizenship**		
Post Office Address								
Post Office Address								
City		**State**		**ZIP**		**Country**		

Name of Additional Joint Inventor, if any:	☐ A petition has been filed for this unsigned inventor							
Given Name (first and middle [if any])			Family Name or Surname					
Inventor's Signature						**Date**		
Residence: City		**State**		**Country**		**Citizenship**		
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Post Office Address								
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09/787397
532 Rec'd PCT/PTO 16 MAR 2001

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